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Dendritic Cells: A Life Story

Jacques Banchereau, Karolina Palucka and Bali Pulendran
Baylor Institute for Immunology Research, Dallas, Texas

DC constitute a complex system of cells which, under different microenvironmental conditions, can induce such contrasting states as immunity and tolerance. The DC heterogeneity is reflected at several levels: (i) precursor populations (ii) anatomical localization (iii) function; particularly with regard to the regulation of B cell proliferation (interstitial DC) and differentiation and the polarization of T cell responses towards type I (DC1) or type II (DC2), and (iv) the final outcome of immune response, i.e. the induction of immunity or tolerance.

DC play a critical role in the immediate reaction to tissue injury and in the shaping of immune response. The emerging finding is the plasticity of the DC system illustrated by (1) specialization of different subsets of DC precursors to respond to different pathogens, virus or bacteria; (2) multipotent differentiation ability of precursor populations; (3) the dual function of DC at distinct stages of differentiation: (i) ability to secrete large amounts of pro-inflammatory and/or antiviral cytokines in the precursor form, and (ii) ability to activate and modulate T cell responses in their mature form; and (4) differential regulation of T cell responses dependent of local cytokine environment.

All these properties permit to develop strategies allowing for manipulation of DC subsets both *in vivo* and *ex vivo* to induce a desired type of immune responses.

Apoptosis

Nina Bhardwaj
Rockefeller University, New York, New York

Cell death by necrosis is typically associated with inflammation, in contrast to apoptosis. We have identified additional distinctions between the two types of death that occur at the level of dendritic cells and which influence the induction of immunity.

Dendritic cells [DCs] but not macrophages efficiently phagocytose apoptotic cells and cross-present viral, tumor and self-antigens to CD8 + T cells. Phagocytosis of apoptotic cells is restricted to the immature stage of DCs, when the DCs express a unique profile of receptors, in particular the alpha v beta 5 integrin and CD36.

To act as potent antigen presenting cells, however, DCs must undergo changes termed maturation. We find that optimal cross-presentation of antigens in tumor cells requires two steps: phagocytosis of apoptotic cells by immature DCs, which provides antigenic peptides for MHC class I presentation, and a maturation signal that is delivered by exposure to necrotic tumor cells, their supernatants or standard maturation stimuli, e.g. monocyte conditioned medium. The mature dendritic cells express high levels of the DC restricted markers CD83, DC-LAMP and the costimulatory molecules CD40 and CD86. Thus dendritic cells are able to distinguish two types of tumor cell death, with necrosis providing a control that is critical for the initiation of immunity.

Antigen Presentation by CD1: a Special Role for CD1a on Langerhans Cells

Michael B. Brenner, Masahiko Sugita, Victor Hsu and Peter Peters
Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

CD1 molecules are a lineage of nonpolymorphic cell surface glycoproteins related in evolutionary origin to the major histocompatibility complex (MHC) antigen-presenting molecules. Five distinct isoforms, CD1a, b, c, d, and e are found in humans encoded on chromosome 1. Remarkably, the antigens presented by CD1 are lipid in nature, in contrast to the peptide fragments of proteins presented by MHC molecules. This is possible because the CD1 antigen-combining superdomain consists of two large hydrophobic pockets that bind lipid tails rather than amino acids side chains. The T cells recognizing CD1 presented antigens are typically CD8 + or CD4–8-cytolytic T cells. These T cells are capable of killing microbe infected dendritic cells, and produce interferon γ, suggesting that CD1a, b, and c restricted T cells that recognize microbial lipid antigens may play an important role in host defense. Separately, T cells directed against CD1d that include the NKT cells are most notable for their ability to produce high levels of both interferon γ and IL-4.

We have found that CD1a molecules, expressed on Langerhans cells and certain other dendritic cells such as in the bronchial epithelium, also are capable of presenting foreign microbial antigens in a CD1a restricted manner. In addition, other T cells recognize CD1a in the absence of added foreign antigens and constitute an autoreactive pool of T cells in the human immune system. CD1b, c and d molecules all contain a cytoplasmic tail tyrosine-based motif that directs their trafficking into the endocytic system. CD1b, in particular, traffics to late endosomes and lysosomes where it colocalizes with MHC class II in acidic compartments. In contrast, we found that CD1a molecules traffic independently and lack the tyrosine based cytoplasmic tail targeting motif. CD1a molecules were localized to early endosomes that participate in the recycling pathway, and efficiently excluded from the deep endocytic system. Functionally, antigen presentation by CD1a is also distinct because it is not dependent on vesicular acidification, in contrast to CD1b or MHC class II. Using immunogold labeling transmission electron microscopy, CD1a was localized to Birbeck granules that are characteristic of Langerhans cells. Together, these studies emphasize the

Langerhans Cells Have Unique Features Illustrating Selective Migration, Antigen Uptake and Routage Capacities

Christophe Caux, Jenny Valladeau, Marie-Caroline Dieu, Odile Ravel, Béatrice Vanbervliet, Alain Vicari, Sem Saeland and Serge Lebecque
Schering-Plough, Laboratory for Immunological Research Dardilly, France

Langerhans cells (LC) are epithelial immature dendritic cells, involved in the regulation of skin and mucosal immune responses. Their origin and specific functions remain areas of investigation. In the present study we illustrate through two examples that LC have unique properties not shared by other immature DC populations.

First, as a way to understand the regulation of the complex DC traffic pattern, we have investigated the chemokine responsiveness of different DC populations. We found that LC type DC, derived from $CD34^+$ HPC, migrate mainly in response to MIP-3α while monocytes derived DC do not. In addition, *in vivo*, the only DC attracted by MIP-3α and expressing its receptor, CCR6, are epithelial LC. Furthermore, the *in vivo* MIP-3α expression is restricted to inflamed epithelium. These observations indicate a specific role for MIP-3α in the recruitment of LC at epithelial surface during inflammation and suggest that other DC populations may lack the capacity to reach the epithelium.

Second Langerin, a novel type II transmembrane C-type lectin, is a LC surface specific molecule not expressed on any other DC population. Langerin functions as an endocytic receptor and delivers its ligand into Birbeck granules but not into class II MHC-rich compartments. Finally, transfection of Langerin cDNA into fibroblastic cells results in a dense network of Birbeck granules. These results suggest that LC may have selective antigen recognition pattern and unique routage of material endocytosed through Langerin.

Finally, both CCR6 and Langerin are lost upon maturation stimuli while CCR7 is induced, allowing the maturing DC to enter the draining lymphatic through 6Ckine and MIP-3α expression. The mature DC, homing in the T cell area, have lost their antigen uptake capacity but acquired costimulatory molecules required to prime naive T cells.

Altogether, these examples illustrate that LC have unique features (origin, location, recruitment, antigen recognition, antigen routage) strongly arguing for specialized functions.

Gene Therapy and Dendritic Cells

Ronald Crystal
Weill Medical College of Cornell University, New York, New York

Dendritic cells (DC), the most potent of the professional antigen presenting cells, can present tumor-associated antigens to the immune system, with consequent generation of tumor-specific cytotoxic T-lymphocytes (CTL) and suppression of tumor growth. Based on the concept that this function can be enhanced by transient adenovirus (Ad) genetic modification of tumors and/or DC with genes relevant to DC function, we have developed a variety of *ex vivo* and *in vivo* strategies to attract DC to tumors and to amplify tumor DC function in antitumor immunity. For example, Ad vectors can be used to transfer chemoattractants for DC to tumors, resulting in the accumulation of DC within the tumor. Alternatively, based on the knowledge that triggering of CD40 on DC by CD40 ligand (CD40L, normally found on activated CD4 + T cells) is important for DC activation, the CD40L cDNA can be transferred to tumor cells, thus forcing the tumor cell to help activate the DC. Strikingly, the CD4 + T cell/DC interaction can be bypassed by using an Ad vector to transfer to CD40L cDNA directly to DC, allowing the DC to express both CD40 (the receptor) and CD40L (the CD40 trigger). In all these strategies, the consequences are generation of tumor specific CTL and suppression of growth of pre-existing tumors. Together with the development of methodologies to purify human DC, it should be possible to move these strategies to the clinic where gene therapy can be adapted to enhancing normal DC function.

Langerhans Cells and Other Types of Dendritic Cells in Tumor Immunity and Tumor Immunotherapy

Stephan Grabbe
University of Münster, Münster, Germany

Due to their potent antigen presenting capacity, dendritic cells (DC) are currently under investigation as immunotherapeutic agents for the induction of antigen-specific, T cell-mediated immune responses. It has been demonstrated that murine Langerhans cells (LC) as well as bone marrow-derived DC can prime naive T cells for the generation of tumor-specific immune responses and induce both prophylactic and therapeutic tumor immunity in a variety of tumor systems. It has also been demonstrated that tumor immunotherapy with autologous human peripheral blood-derived DC can result in partial or even complete remission of metastatic melanoma and plasmacytoma in up to 30% of the patients treated. Nevertheless, a number of variables still need to be determined to optimize the therapeutic efficacy, among which are the DC maturation state, antigen loading and type of tumor antigen, site, dose and interval of DC application. In order to address some of these variables, we tested murine bone marrow-derived DC of different maturation stages for their capacity to induce protective or therapeutic tumor immunity against the poorly immunogenic murine squamous cell carcinoma, KLN205. For this purpose, naive or tumor-carrying mice were injected subcutaneously 2 times at weekly intervals with tumor antigen (TA)-pulsed DC. Whereas immunizations with immature DC (generated by culture in GM-CSF only) did not retard tumor growth when compared to untreated control animals, tumor size and incidence was significantly reduced after injection of mature, TA-pulsed DC (generated by culture in GM-CSF + IL-4 and subsequent activation with CD40L). The immunotherapeutic efficacy of these DC correlated with their capacity to migrate into regional lymph nodes. Surprisingly, it appeared that the timepoint of tumor antigen loading was not critical for their capacity to induce tumor immunity *in vivo*. A combination of DC-immunotherapy and systemic administration of GM-CSF plus IL-2 or CD40L augmented the therapeutic efficacy. These preclinical studies may help to improve DC-immunotherapy protocols for the treatment of human malignancies.

Neuropeptides and Langerhans Cells

Richard D. Granstein
Weill Medical College of Cornell University, New York, New York

Anecdotal evidence suggests regulation of immunity and inflammation in the skin by products of nerves. For example, skin diseases including psoriasis and atopic dermatitis are said to worsen with anxiety. In support of this possibility, experiments in which capsaicin has been used systemically to deplete animals or applied topically to deplete skin sites of sensory neuropeptides lead to increased contact and delayed-type hypersensitivity responses. This suggests that the net influence of sensory neuropep-tides is inhibitory, at least within the skin. The possibility that epidermal Langerhans cells (LC) are influenced by products of nerves comes from the finding that these cells are frequently in anatomic association with epidermal nerves. LC have been shown to have receptors for several neuropeptides including calcitonin gene-related peptide (CGRP), gastrin-releasing peptide (GRP), pituitary adenylate cyclase-activating peptide (PACAP), vasoactive intestinal polypeptide (VIP) as well as adrenergic receptors. In a number of assays, CGRP inhibits antigen presentation by populations of epidermal cells, suggesting an effect on LC function. Experiments with dendritic cell lines and macrophages suggest that CGRP may inhibit the upregulation of CD86 and alter expression of immunomodulatory cytokines in a manner that decreases their ability to stimulate cell-mediated immune responses. Other studies have suggested a role for CGRP in ultraviolet radiation-induced immunosuppression. Recent experiments also suggest that adrenergic agents regulate cytokine expression by dendritic cells and inhibit antigen presentation *in vitro* by epidermal cells. As a whole, these findings support the concept that nerves play a modulatory role in immune reactivity within the epidermis by release or nonrelease of afactors that modify LC function.

Cell Biology of Dendritic Cell Function

Ira Mellman and Ralph Steinman
Ludwig Institute for Cancer Research, Yale University School of Medicine, New Haven, CT

Although the importance of dendritic cells (DCs) in initiating the immune response has been well established, we are only now beginning to understand the cellular mechanisms by which DC function is controlled. We have investigated the events associated with the conversion of immature DCs in peripheral tissues from sentinels adapted for antigen capture to mature DCs in lymphoid organs adapted for T cell stimulation. A remarkable array of mechanisms act synergistically and in a variety of DC types, including Langerhans cells differentiated from mobilized CD34+ precursors. MHC class II molecules are diverted from lysosomal compartments to the cell surface both at least two strategies. Newly synthesized MHC class II is re-routed to the plasma membrane in maturing cells by enhancing the efficiency of cathespin S-mediated invariant chain cleavage by a down regulation of the cathespin S inhibitor cystatin C. Previously synthesized MHC class II is physically removed from lysosomes using a novel mechanism involving the formation of distinctive 'class II vesicles' that comprise a type of secretory vesicle possibly unique to DCs. Endocytosis is down regulated upon maturation, limiting further antigen uptake, by down regulating the activity of the Rho family GTPase Cdc42. Perhaps most striking however, is the fact that DCs regulate the very ability to generate immunogenic peptide-MHC complexes intracellularly. Complex formation, even between MHC class II and antigen molecules residing within the same lysosomal structures, is tightly linked to the receipt of a maturation stimulus such as LPS or TNFα. The complexes, are then selectively sequestered into 'class II vesicles', remarkably along with MHC class I and B7 costimulatory molecules, and delivered to the plasma membrane where they appear to exist as a complex or differentiated microdomain. Conceivably, these clusters of MHC and costimulators underlie the ability of even fixed DCs to stimulate naïve T cells.

Cytokine Gene Therapy of Cancer Using Fibroblasts or DCs

Michael T. Lotze, Hideaki Tahara, Walter Storkus, Hideho Okada, Paul Robbins, William Chambers, Kazumasa Hiroishi, Shin-ichi Egawa and Siamek Mohammadi
University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

One of the central problems in cancer therapy is to identify agents which selectively target the tumor and which have limited impact on the normal cells of the host as well as finding means to deliver them. Cytokine gene therapy obviates many of the substantial problems associated with systemic cytokine delivery, accomplishing the central goals of Immunology: (1) Recruiting naïve immune effectors to the tumor site and activating them; (2) Promoting antigen processing and presentation to T-cells; (3) Eliciting specific T-cells which can cross the endothelial barriers within tumors; (4) Regulating angiogenesis within the tumor; and (5) Maintaining a long-lived memory T-cell response. Dendritic cells represent important delivery means to enable delivery, recruitment of immune cells, promote antigen processing and presentation, regulate angiogenesis and maintain immune effectors in the tumor microenvironment. We have completed Phase I/II studies using IL-4 gene therapy approaches (18 patients) or IL-12 (32 patients to date) with delivery by transfected autologous fibroblasts. Responses were observed in melanoma patients (3/8 and 3/6, respectively). Associated findings included endothelial activation and lymphocyte/DC infiltrate. Novel regulated expression vectors are in development as well.

Dendritic Cell Gene Therapy. Tumor derived peptides can sensitize immune effectors for tumor lysis when pulsed onto dendritic cells. The ability to introduce genes into IL-4 and GM-CSF expanded dendritic cells allows exploration of their use when transfected with tumor antigens, costimulatory molecules or alternatively cytokines such as IL-12 prior to administration. Murine models suggest that cytokine transfection can markedly enhance the efficacy of such approaches. Expression of CD40L or IL-12 using plasmids in DC's appears to enhance the antitumor efficacy of adoptively transferred cells pulsed with specific tumor peptide. DCs play important roles as antigen presenting cells but are likely also important in T-cell survival in the periphery, as sources of antiangiogenic agents, and as portals for entry of pathogens such as HIV. The major problem associated with current adoptive therapies is that they utilize T-cells, which have been expanded from tumor sites where they are admittedly ineffective. Developing new T cell (and potentially B cell) reactivity to tumor is a major goal of DC-based strategies. We performed a clinical protocol in which 28 HLA-A2+ patients with melanoma had peripheral blood derived dendritic cells cultured with GM-CSF and IL-4 for 5–7 days, pulsed with synthetic melanoma peptides derived from the MART1/Melan A, gp100, and tyrosinase melanosomal proteins. The average number of DCs administered was 10^6 each week. Two patients have sustained a CR and one has had a PR. Two patients have been treated with G-CSF (780 μg/d $\times$ 5d) mobilized macrophages and up to 125×10^6 cells administered SQ or IV without major toxicity including an HLA-A2 negative patient with RCC. Unanswered questions are those related to the number of tumor cells required, the optimum strategy for delivery (timing, route, method, and type of natural antigen – following *in vitro* manipulation to induce apoptotic death), and the appropriate cytokine stimulation. In murine models administration of IL-12 transfected DC is associated antitumor effects and induction of TH1 type systemic antitumor immunity.

Genetic Immunization Technology Applied to Manipulating Dendritic Cells

Stephen Albert Johnston, Bao-Xi Qu, Kathy Sykes, Akira Takashima, Michael McGuire and Laura Timares
University of Texas-South-western Medical Center, Dallas, Texas

Genetic immunization not only holds promise as a method to deliver vaccines, but also as an effective tool to manipulate and explore the immune system. I will provide several examples of this relevant to dendritic cells (DC).

1. We have developed a new genomics technology termed Linear Expression Elements that allows screening genomes for phenotypes without cloning. We have applied this to screen the parapox genome for genes that affect DC migration, with an unexpected finding.

2. We have developed an inducible vaccination system. This permits vaccination on day 1 and then induction of an immune response at any desired time later. This system was used to finally resolve whether migrating DC, expressing plasmid genes are responsible for the immune response in genetic immunization.

3. We have proposed that restricting antigen expression to particular cells (e.g. APC vs. non-APC) might have either a stimulatory or tolerizing effect on the immune response. We have tested this idea with plasmids whose expression is limited largely to dendritic cells or keratinacytes. We report evidence that supports this concept.

Human Type 2 Dendritic Cells in Innate and Adapted Immunity

Yong-Jun Liu, Vassili Soumelis and Norimitsu Kadowak
DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, California

Two immediate dendritic cell precursors can be isolated from human blood: Monocytes, the myeioid DC1 presursors and CD4+CD11c-plasmacytoid cells, the 'lymphoid DC2 precursors.' Although both DC1 and DC2 strongly stimulate T-cells, they, respectively, induce TH1 vs. TH2 differentiation. DC2 precursors were found to be the elusive natural IFN-producing cells. These cells produce up to 1000 time more IFN-alpha in response to viral stimulation. Thus the human type 2 dendritic cell lineage play critical function in both innate and adapted immunity.

How Dendritic Cells Handle Bacteria

Paola Ricciardi-Castagnoli
University of Milano-Bicocca, Milan, Italy

There is increasing evidence that dendritic cells bridge innate and adoptive immune responses. This ability is the result of the unique plasticity of dendritic cells which allows mature DC to undergo complete transcriptional re-programming when they encounter microorganisms.

Using homogeneous immature mouse dendritic cell line derived from mouse spleen and bone marrow, we have analyzed the kinetic of the interaction between DC and Gram + or Gram- bacteria. Transcriptional analyzes has been evaluated first using the Affymetrix gene chips which display 6400 mouse genes. The kinetic of receptor expression, cytokines and chemokine expression was evaluated after bacteria uptake. In addition, the kinetic of the presentation of bacteria antigens with Class I and II molecules has been analyzed. As a whole the results suggest that, upon bacteria uptake, immature DC undergo functional re-programming which allows them to link adoptive with acquired immunity.

Dendritic Cells and HIV

Melissa Pope
Rockefeller University, New York, New York

The importance of dendritic cells [DCs] in HIV infection has been highlighted by the considerable *in vitro* studies which have demonstrated how immature DCs produce detectable levels of infectious virus, while mature DCs carrying low level infection more efficiently amplify virus replication in the presence of $CD4^+$ T cells. Although difficult to document *in situ*, it has been suggested that immature DCs at the body surfaces are exposed to virus following mucosal transmission and then carry virus to the lymphoid tissues, where the now matured DCs rapidly spread infection to surrounding permissive $CD4^+$ T cells. However, it is critical to establish how to exploit the DC to execute its normal immunological function of inducing potent antiviral immunity, rather than fostering growth and expansion of the virus.

To do this we have employed the SIV-macaque system. This animal model, that closely mimics the human system, allows us to [i] accurately dissect the requirements for virus replication in different DC environments *in vitro* and *in vivo*, [ii] access tissues from infected animals to ascertain the contribution of DCs during acute and chronic stages of infection via different routes, and [iii] directly target DCs for the induction of antiviral immunity. Characteristic DCs can be isolated from macaque tissues and blood. Mature DC-T cell mixtures isolated from different anatomical locations promote SIV replication *in vitro*. However, a 2–4 day delay in the kinetics of SIV replication occurs when immature DCs encounter naïve $CD4^+$ T cells, compared to that seen in the presence of immature DCs and memory T cells or mature DCs with either T cell subset. Furthermore, an attenuated form of SIV lacking the *nef* gene, SIV delta nef, cannot replicate well in the immature DC-$CD4^+$ T cell milieu, while it replicates normally in the presence of mature DCs. These data have important implications for the conditions that are required to expedite the spread of virus in various DC-T cell environments, and the specific mechanisms involved are under investigation. Furthermore, we believe that the compromised replication of SIV delta nef in the immature DC-T cell mixtures may contribute to the ''vaccine effect'' of this attenuated SIV isolate *in vivo*. *In vivo* studies are underway to investigate the ability of DCs to transmit infection vs. induce virus-specific immunity when loaded with various forms of live and killed viral antigen preparations prior to re-injection into the donor animal, for the potential application of DCs in vaccine and therapeutic strategies.

Development and Functions of Dendritic Cells

Ralph M. Steinman
Rockefeller University, New York, New York

5 stages describe the life history of DCs: proliferating progenitor, nonproliferating precursor, immature DC, mature DC and apoptotic death. Within this framework, there may be more than one differentiation pathway, e.g. epidermal Langerhans cells (LCs) vs. dermal DCs. Factors that control each of the 5 stages are being found. Flt-3 ligand stimulates progenitors to yield many nonproliferating precursors. Precursor monocytes can be differentiated into DCs by a 2-step culture that begins with exposure to GM-CSF and IL-4, followed by a maturation stimulus. Monocytes also differentiate to DCs during reverse transmigration across endothelium. Ex vivo antigen-pulsed, monocyte-derived DCs efficiently induce immunity in humans. LCs use multidrug resistance receptors during migration from skin. The lifespan of mature DCs is prolonged if the cells are treated with TNF molecules like CD40L and TRANCE. When DCs die *in vivo*, they are processed by other DCs in lymph nodes.

A critical control point in the life history of the DC is maturation, first encountered in studies of LC development. During maturation, DCs begin to make IL-12 and resist the suppressive effects of IL-10, express several costimulator molecules, and change their chemokine receptors in a way that facilitates homing to T cell areas. Experiments with Dr Inaba's lab in Kyoto and Dr Mellman's in New Haven have uncovered new consequences of DC maturation. MHC class II-peptide complexes form much more efficiently if DCs receive a maturation stimulus following the uptake of protein antigens into MHC II-rich compartments. Maturation of mouse DCs markedly enhances their capacity to stimulate naïve T cells, a finding that should impact in the use of DCs as adjuvants for immunotherapy in humans.

Antigen Presenting Cells and Tolerance

J. Wayne Streilein, H. Niizeki, I. Kurimoto and T. Kitazawa
Schepens Eye Research Institute, Harvard Medical School, Boston, Massachusetts

Hapten application to normal skin results in contact hypersensitivity (CH) induction, a process that depends upon cutaneous antigen presenting cells (APC, Langerhans cells, dermal dendritic cells). If skin is first exposed to ultraviolet B radiation (UVR), hapten application induces tolerance rather than CH, a process that also involves cutaneous APC. One type of tolerance, induced by hapten painted on skin immediately after acute, low dose UVR, is hapten-specific, arises from tolerance-conferring, bone marrow-derived dermal cells, and is mediated by suppressor T cells. In this tolerance, UVR releases CGRP from cutaneous nerve terminii, CGRP induces dermal mast cells to release IL-10, and IL-10 acts directly on hapten-bearing dermal APC. Upon arrival in draining lymph nodes, IL-10-exposed APC secrete IL-10 rather than IL-12, and activate T cells that suppress CH. A second type of UVR-promoted tolerance prevents CH to all haptens, is systemic and mediated by suppressor T cells, and is associated with a profound APC defect in secondary lymphoid tissues, but not skin. The systemic APC defect arises from IL-10 produced by UVR-exposed keratinocytes in sufficient quantities to appear in the blood. APC treated *in vitro* with IL-10 display a profound deficit of IL-12 production and CD40 expression, and when pulsed with antigen and used to activate naive T cells, the responding cells neither express CD40 ligand nor produce IFN-g, IL-4 or IL-10. It is proposed that the final common pathway to UVR-dependent tolerance is via skin-derived IL-10 (keratinocytes, mast cells, dendritic cells) which modifies the functional properties of cutaneous APC and/or APC in secondary lymphoid organs, leading to activation of regulatory rather than effector T cells.

Antigen-Specific T Cell Elimination *In Vitro* and *In Vivo*

Jack L. Strominger, Kirsten Falk and Olaf Rötzschke
Harvard University, Cambridge, Massachusetts

We previously reported that the oligomerized T cell epitope HA306–318 'superactivated' the proliferation of HA306–318-specific HLA-DR1-restricted human T cell lines and clones, i.e. the maximum proliferative response was observed at 3 logs lower concentration of oligomer (5 ng/mL) than of peptide (5 μg/mL) (1). In continuation of this work, the effect of higher doses of antigens was explored (2). With oligomer proliferation rapidly diminished and reached the baseline at 0.5 μg/mL, while low level proliferation was still observed at 50 μg/mL of peptide, again representing a 3 log difference in the concentration required to achieve 'high zone tolerance.' At the high zone with oligomer the TCR of responding T cells was internalized and cell death occured, as judged by Trypan blue exclusion or by DNA degradation, while neither of these phenomena was observed with peptide and proliferation resumed by 10–14 days. Next, the *in vivo* effects of an oligomer were examined utilizing as a model the induction of EAE in SJL mice with PLP139–151 (C140S). A single i.v. dose of PLP139–151 oligomer at 7 days after immunization with peptide in CFA prevented the appearance of disease in the control at 10 days (3). Relapses occured at 40–45 days, but a second intravenous dose of oligomer at 40 days prevented relapse. Moreover, if mice were given two i.v. injections of oligomer at days −7 and −3, disease could not be induced by immunization with peptide in CFA at day 0. This result represents a form of vaccination against EAE. Similarly, EAE induced in mice by MBP86–100 in CFA was prevented by i.v. injection of the corresponding oligomer. Both the *in vitro* and the *in vivo* effects of the oligomers were shown to be antigen (peptide)-specific.

Analysis of Differences Between Dendritic Cells and Macrophages: Toward Development of Dendritic Cell-Based Immunotherapies

Akira Takashima
University of Texas, South-western Medical Center, Dallas, Texas

Dendritic cells (DC) differ from other antigen presenting cells (e.g. macrophages) in their tissue distribution and surface phenotype, as well as the capacity to migrate from epithelial tissues into lymph nodes and deliver activation signals to naive T cells. Langerhans cells (LC), which are skin-resident, immature members of the DC family, play crucial roles in the initiation of T cell-mediated immune responses to a variety of skin relevant antigens. Our major objective has been to develop new immunotherapies that are designed to manipulate LC function experimentally. Recently, we developed a small peptide inhibitor of LC migration. Using a phage display strategy, we identified a 12-mer peptide (termed 'Peptide 1') that binds, and blocks the function, hyaluronan (HA), which is known to serve as an adhesive substrate for LC migration. Local injection of Peptide 1 in mice before topical application of DNFB blocked almost completely the emigration of LC from the epidermis to the draining lymph node, where antigen presentation takes place. Peptide 1 is a new strategy, the initial event of LC-dependent initiation of cellular immune responses. Under the hypothesis that LC that are engineered to over-express a death ligand would deliver apoptotic signals, instead of activation signals, to T cells, we have introduced CD95L cDNA into our long-term LC line XS106. The resulting 'killer' LC clone expressed functionally active CD95L on the surface as assessed by its ability to kill CD95-expressing targets. Importantly, anti-CD95L mAb reverted the killer LC into conventional LC that deliver T cell-stimulatory signals. *In vivo* administration of OVA-pulsed killer LC into mice, either before or after sensitization, resulted in marked suppression of delayed-type hypersensitivity responses to OVA. Likewise, DNFB-pulsed killer LC suppressed contact hypersensitivity responses to DNFB. The killer LC technology represents an entirely new immunosuppressive therapy that is designed to eliminate only the pathogenic T cells in an antigen-specific manner.

Tgfβ1 and Langerhans Cell Development

Mark C. Udey
Dermatology Branch, National Cancer Institute, Bethesda, MD

The pleiotropic cytokine transforming growth factor β1 (TGFβ1) is well known as a negative regulator of inflammation. Thus, it is not surprising that the most dramatic abnormality in two independent strains of TGFβ1 knockout mice is a multi–organ inflammatory syndrome that is lethal within the first month life. While studying Ep-CAM, a putative homotypic adhesion molecule that is present on some murine dendritic cells *in vivo* and whose expression by dendritic cells *in vitro* is regulated by TGFβ1, we determined that TGFβ1 knockout mice are devoid of Langerhans cells. Subsequent studies revealed that the Langerhans cell deficiency could be dissociated from the lethal inflammatory syndrome and that the requirement of Langerhans cells for TGFβ1 was not cell autonomous. Investigations by others have demonstrated that TGFβ1 also plays an important role in the development of human Langerhans cell-like cells from immature progenitors *in vitro*. Recently it has also been determined that TGFβ1 promotes development of Langerhans cell features by human peripheral blood monocytes. The present discussion will briefly review what is known about regulation of Langerhans cell development by TGFβ1 in mice and humans and include speculation about possible mechanisms by which this important cytokine influences dendritic cell ontogeny and function.